Copy when uncertain: lower light levels increase trail pheromone deposition and reliance on pheromone trails in ants

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Copy when uncertain: Lower light levels increase trail pheromone deposition and reliance on pheromone trails in ants

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Abstract

Animals may gather information from multiple sources, and these information sources may conflict. Theory predicts that, all else being equal, reliance on a particular information source will depend on its information content relative to other sources. Information conflicts are a good area in which to test such predictions. Social insects, such as ants, make extensive use of both private information (e.g. visual route memories) and social information (e.g. pheromone trails) when attempting to locate a food source. Importantly, eusocial insects collaborate on food retrieval, so both information use and information provision may be expected to vary with the information content of alternative information sources. Many ants, such as *Lasius niger*, are active both day and night. Variation in light levels represents an ecologically important change in the information content of visually-acquired route information. Here, we examine information use and information provision under high light levels (3200 lux, equivalent to a bright but overcast day), moderate light levels simulating dusk (10 lux) and darkness (0.007 lux, equivalent to a moonless night). Ants learn poorly, or not at all, in darkness. As light levels decrease, ants show decreasing reliance on private visual information, and a stronger reliance on social information, consistent with a 'copy when uncertain' strategy. In moderate light levels and darkness, pheromone deposition increases, presumably to compensate for the low information content of visual information. Varying light levels for cathemeral animals provides a powerful and ecologically meaningful method for examining information use and provision under varying levels of information content.

Key words

ants; information conflict; information use strategies; light levels; pheromone deposition; route learning

Introduction

Strategic information use is critical to the success of many animals. Animals must, for example, decide whether to explore new options, exploit the knowledge they already have, or use information gleaned from or sent by other animals about potential options (Grüter & Leadbeater, 2014; Laland, 2004; Leadbeater & Dawson, 2017). Two important classes of information for animals are private information and social information. Private information sources cannot be accessed by others, and include genetic information, internal states, and importantly, memories. Social information is information gathered from observation of, or interaction with, other animals or their products (Dall, Giraldeau, Olsson, McNamara, & Stephens, 2005; Heyes, 1994). These may be cues, such as indications of conspecifics having recently been in a particular location (Worden & Papaj, 2005; Wüst & Menzel, 2017), or intentionally produced social signals, such as the waggle dance of honey bees or pheromone trails deposited by ants (Czaczkes, Grüter, & Ratnieks, 2015; Grüter, Balbuena, & Farina, 2008). Unless stated otherwise, in this work we follow Shannon (1948) in defining information as something which reduces uncertainty about the state of the world. An information source is something which could potentially offer information, even if in a specific situation it may not. Thus, 'memory' is a private information source, but if there is nothing stored in the memory, it cannot provide information. Individuals can choose to use an information source by using its content to change their estimate about the state of the world (e.g. pay attention to a pheromone trail when attempting to locate a food source), but may choose not to.

While much research effort has been focussed on assessing whether to exploit available information or innovate by gathering (often costly) new information (Danchin, Giraldeau, Valone, & Wagner, 2004; Laland, 2004), once information is gathered animals must decide how to use multiple information sources. Matters are complicated when conflicts arise between information sources. When this occurs, one option is to produce and follow a weighted intermediate value (von Thienen,

Metzler, & Witte, 2016; Wehner, Hoinville, Cruse, & Cheng, 2016; Wystrach, Mangan, & Webb, 2015). For example, if one information source suggests a goal is North-West, and a second more preferred one suggests it is North-East, the animal might head North-North-East – as is seen, for example in desert ants (Wystrach et al., 2015). However, sometimes an intermediate response is not possible, for example when deciding between two feeding locations. Alternatively, a hierarchy of information sources can be employed, with one type of information being exclusively used until it is not available, after which others begin to be employed; for example, honeybees use the sun's azimuth as a compass direction on cloudless days, but when the sun is not visible they rely on polarised light for this information (Dyer & Could, 1983; von Frisch, 1949). A more nuanced strategy is to weigh up the usefulness or information richness of different information sources, and follow the best one (Koops, 2004). It is possible that apparent hierarchical information use is in fact weighted intermediate use, with one option being overwhelmingly weighted above another.

Social insects, such as ants and bees, offer an unique system in which to study information-use strategies (Grüter & Leadbeater, 2014; Leadbeater & Dawson, 2017). Firstly, social information use is likely to be very well developed in this group. More fundamentally, however, in many aspects of information use by social insects, the interests of individuals are aligned within a colony. For example, we do not expect to see individuals from the same colony attempting to monopolise a resource. This should lead to full honesty in communication, making social signals more valuable, as there is no chance of deception, although information can still become outdated or resources overexploited (Beckers, Deneubourg, Goss, & Pasteels, 1990). Critically, it also means that information providers, rather than being exploited, are benefiting from providing information. This in turn is expected to result not only in strategic information use in the receiver, but also in strategic information provision by the signaller. The context in which social insects choose to actively produce social information can be as informative as the context in which they choose to respond to it (Grüter & Czaczkes, 2019; Grüter & Leadbeater, 2014). For example, ants which are more likely to make a mistake (and thus presumably are more uncertain) have been found to deposit less pheromone

(Czaczkes & Heinze, 2015). Conversely, ants which initially make a wrong choice at a bifurcation when outgoing to the food source, and then correct their mistake to eventually find the food source, deposit more pheromone than ants which initially make a correct decision (Czaczkes, Grüter, & Ratnieks, 2013; Czaczkes & Heinze, 2015). Ants from colonies in which individuals show poorer abilities to learn food locations, tend to deposit more pheromone when returning from a food source (Pasquier & Grüter, 2016). Reports also describe ants depositing pheromone to lower-quality resources only in the dark (Cammaerts & Cammaerts, 1980).

These attributes should, in principle, strengthen the effect of social information on the behaviour of social insects. It is therefore surprising that in most cases in which conflict between social signals and private information have been studied, ants and bees predominantly follow their own memories (Almeida, Camargo, Forti, & Lopes, 2018; Aron, Beckers, Deneubourg, & Pasteels, 1993; Cosens & Toussaint, 1985; Fourcassie & Beugnon, 1988; Grüter et al., 2008; Grüter, Czaczkes, & Ratnieks, 2011; Harrison, Fewell, Stiller, & Breed, 1989; Quinet & Pasteels, 1996; Rosengren & Fortelius, 1986; Stroeymeyt, Franks, & Giurfa, 2011; Traniello, 1989). While this is not a universal pattern (Aron et al., 1993; Middleton, Reid, Mann, & Latty, 2018; Vilela, Jaffé, & Howse, 1987), it is nonetheless striking, as one might *a priori* expect social insects to prioritise social information.

One reason for ignoring social information is that social information is often information-poor when compared to memories. For example, while the number and rate of waggle runs, and intensity of pheromone deposition, increase with resource quality, the inter- and intra-individual variation for set resources qualities are very large (Seeley, Mikheyev, & Pagano, 2000; Wendt, Strunk, Heinze, Roider, & Czaczkes, 2019). By contrast, private memories of a food sources' quality are very accurate, with *L. niger* being able to distinguish between, and reliably follow cues to, sucrose sources differing by just 0.1M (de Agro et al, in preparation). It is possible that insects are attempting to follow a 'copy if better' strategy (Laland, 2004), but without accurate quality information, rarely copy. Using a 'copy if better' strategy implies exploiting private information

(memory) until social information reveals the presence of better options. And indeed, when unambiguous quality information about a better food source is provided, *Lasius niger* ants switch from following memories to following pheromone trails (Czaczkes, Beckwith, & Horsch, 2018). Reversals in information use, from reliance on memories (private information) to reliance on social signals (social information), allow us to understand the strategies animals use when choosing information sources.

Another important reversal in social information use was reported by Beugnon & Fourcassie (Beugnon & Fourcassie, 1988; Fourcassie & Beugnon, 1988). During daylight hours and in a well-lit laboratory, *Formica pratensis* wood ants followed memories over pheromone trails. However, at night their behaviour reversed, and they were found to preferentially follow pheromone trails. While this study was purely descriptive, and did not involve experimental manipulation of light levels, it nonetheless suggested that ants may be following a 'copy when uncertain' strategy, only relying on chemical signals when memories are unavailable or unreliable. As visual information becomes more scarce one expects a heavier reliance on olfactory information, and indeed such a pattern is found in brain anatomy of congeneric diurnal and nocturnal species of the Australian bull ant, Myrmecia; the former develop larger optic lobes and the latter larger olfactory neuropils (Sheehan, Kamhi, Seid, & Narendra, 2019). Copy when uncertain strategies have been previously reported in other social insects in other contexts, such as flower choice in bumblebees (Smolla, Alem, Chittka, & Shultz, 2016) and during nest relocation in rock ants (Stroeymeyt, Giurfa, & Franks, 2017)

The use and provision of information by animals under different light regimes offers a promising means of studying information conflict and information use strategies. Variation in light levels over many orders of magnitude is a challenge many animals have to cope with every day-night cycle. This variation in light levels results in strong variation in the certainty of visual route memories – the main source of navigational information for many ants (Collett, Chittka, & Collett, 2013; Evison, Petchey, Beckerman, & Ratnieks, 2008; Graham & Cheng, 2009). Here, we study the use and

provision of social information (i.e. following and deposition of pheromone trails) under different light levels in the ant *Lasius niger*. Under high light levels, *L. niger* preferentially follow private route memories over pheromone trails, even if the pheromone trails are very strong (Czaczkes et al., 2018; Grüter et al., 2011). We first confirm that route memories in *L. niger* are based solely on visual cues at high light levels (as reported by Evison et al., 2008). We then ask whether *L. niger* foragers modulate their pheromone deposition (social-information production) in response to different light levels. Finally, we assess their preference for private information (memories) over social information (pheromone trails) at different light levels.

Methods

Study Species

Colony fragments (henceforth 'colonies') of the black garden ant, *Lasius niger*, were collected from Falmer in East Sussex, UK. Each colony was housed in a plastic container (30 × 30 ×10 cm high) with a plaster of Paris base containing a circular nest cavity constructed from plaster of Paris (13.5 cm diameter × 1.5 cm high) and covered by a disc of dark card. All colonies were queenless with 1,000 – 3,000 workers and small numbers of brood, each colony stemming from a different wild queenright colony. Queenless colonies readily forage, produce trails and are commonly used in behavioural experiments (Dussutour, Fourcassie, Helbing, & Deneubourg, 2004; Evison et al., 2008), remaining viable for 18 months or more. Colonies regulate the ratio of foragers and intranidal workers, ensuring a good mix of both. The ants were fed three times a week on a Bhatkar mix (Bhatkar & Whitcomb, 1970), with *ad libitum* access to water. Colonies were maintained at ambient laboratory light and temperature levels, and thus experienced a c. 12:12 light/darkness cycle. Data were collected throughout the year. To ensure foraging motivation, feeding was stopped 4 days prior to experimentation.

General experimental design

Following the method of Grüter et al. (2011) we constructed a foraging trail as shown in Figure 1. A white cardboard bridge (20 × 2 cm) connected the colony container to a transparent polycarbonate plastic T-maze covered with white paper. The stem of the T was 15 cm long and each branch was 11 cm long, with a consistent width of 2 cm. Experiment 1 was run in a small windowless room with an ambient temperature of 22°C. Experiments 2 and 3 were carried out in a small room containing various items of lab equipment and furniture which served as visual landmarks for the foraging ants. We used 3 light levels in our experiments: bright light (3200 lux, emulating a cloudy but bright day), moderate light (10 lux, emulating dusk), and darkness (0.0007 lux, emulating a moonless night). Light intensity was measured repeatedly throughout the experiments using a photometer (LI-COR inc; model LI-188B) to ensure illumination was consistent within treatment replicates. These luminances were chosen to reflect normal daylight, crepuscular light, and a moonless night, respectively. A portable halogen work light (IP 44; model NXS-500P) with a 500 w halogen bulb was used to provide high intensity illumination for the bright-light treatment and a floor lamp with a 230 w linear halogen bulb and dimmer switch (Dar; model OPU 4946) provided illumination for the moderate-light treatment. In the moderate light and darkness experiments, red light was used to provide illumination for experimental working and behavioural observations, but this long wavelength illumination was not detectable by the ants; as in humans, most insects have trichromatic vision [UV, blue and green in the case of insects; (Briscoe & Chittka, 2001), but see (Yilmaz, Dyer, Rössler, & Spaethe, 2017) for evidence of bichromatic vision in an ant]. However, their visible spectrum is shifted towards shorter wavelengths than ours (Menzel, 1979; Yilmaz et al., 2017); for example the spectral sensitivity maxima (λ_{max}) for the ants *Atta sexdens* and *Camponotus blandor* are 500 nm and 570 nm, respectively (Martinoya, Bloch, Ventura, & Puglia, 1975; Yilmaz et al., 2017), and thus considerably shorter than the 700 nm found in humans (Autrum, 1968). To provide pure red light, a sleeve created from 2 ply corrugated cardboard was tightly fitted over the hood of an angle poise lamp with a 60 w bulb. Two 50 mm square 665 nm long pass filters (Schott; model FRG-66550) were slotted tightly together into

a hole cut in the centre of the cardboard hood so that, when switched on, the lamp only provided red light.

Experiment 1: Does the frequency of pheromone deposition change with light level?

Ants were allowed to locate and feed on a drop of 1M sucrose solution, randomly allocated to the end of the left or right branch of the T maze (Fig. 1A). A 5 cm long section of paper, located just before the branches of the T, was marked by lines at either end, and a video camera (Sony; model HDR-XR520) was positioned to record, from the side, all pheromone laying behaviour of ants walking along this designated section (Fig. 1A). This section was chosen because ants were observed to regularly deposit pheromone near the junction and for ease of monitoring. The low lux camera setting was used for the moderate light and darkness treatments. Due to the lower video quality in this setting, it is possible that pheromone deposition estimates in the moderate and dark treatment are underestimates. Depending on foraging activity of the colony, the first 8-12 ants which reached the food source and began to feed were marked with a dot of grey acrylic paint (the most discernible colour under IR light). All unmarked ants were removed from the bridge and T maze. Further access to the setup was controlled by raising or lowering the bridge. The marked ants were allowed to find their way back to the nest, return to the food source, and then once again return towards the nest. Only marked ants were allowed to re-enter the setup by only lowering the bridge when marked ants were present. Unmarked ants that climbed the bridge were gently brushed off. We thus tested batches of 8-12 ants simultaneously. Marked ants were removed after passing through the observation section on this final trip. Thus, a maximum of 3 journeys were recorded for each ant: the first return to the nest, the first return to the food, and the second return to the nest. When analysing the videos we assumed that an ant deposited a drop of pheromone each time we saw it clearly curve and dip its gaster to the surface (Beckers, Deneubourg, & Goss, 1992). The experiment was carried out under the three different lighting regimes using six colonies.

Experiment 2: Effect of illumination on information use choice

To test whether reliance on trail pheromones increases at lower light levels, foraging ants were presented with a conflict between their own route memory and a pheromone trail at a T junction at the three different light levels. Following Grüter et al. (2011), a pheromone trail was created by allowing ants to freely forage on a drop of 1M sucrose situated on the T maze before the bifurcation (Fig. 1B). A piece of paper (section X in Fig. 1B; 10 × 2 cm) was placed directly before the food source with a section of it (4 × 2 cm) covered by an additional piece of paper (segment Y). This ensured that the covered section beneath segment Y remained free from pheromone deposited by ants leaving and returning to the food source. A consistent pheromone trail strength was achieved by ending foraging once 35-40 pheromone depositions had been recorded. The maximum time allowed for trail establishment was 20 min; if the minimum number of depositions was not reached in this time, the experiment was terminated.

Ants were then given an opportunity to learn a food location by placing a 1M sugar solution source on the end of a randomly selected branch of the T maze and allowing the ants to find the food source via the bridge. Feeding ants were marked with a dot of grey acrylic paint and allowed to return to the nest. At high motivation levels such as these, 75-80% of *Lasius niger* foragers take the correct arm of an unmarked T maze at normal levels of illumination after only one visit (Grüter et al., 2011; Oberhauser, Koch, & Czaczkes, 2018). Before these marked ants left the nest to find the food source again, section X was transferred to the bifurcation of the T maze (Fig. 1C) with the pheromone marked side placed on the branch opposite to where the food source had initially been situated. The covering segment Y was removed so that the bifurcation now had two new arms, only one of which was marked with pheromone. The decisions of the returning marked ants were then recorded. The maximum time allowed for memory development and subsequent decisions by the ants was 30 mins, giving a total maximum experimental time of 50 min, when including trail establishment, which corresponds to the mean trail-lifetime (time until a pheromone deposition stops influencing behaviour) reported for *Lasius niger* (Beckers, Deneubourg, & Goss, 1993; Evison et al., 2008). Decisions were recorded for

ants from nine colonies. One of the colonies was previously used in experiment 2. All colonies stemmed from different wild queenright colonies.

Experiment 3: Is memory based solely upon visual cues?

The aim of this experiment was to investigate whether ants could develop a route memory in the absence of visual cues. As in experiment 2 the nest was connected to the T maze by a cardboard bridge and a 1M sucrose solution was placed at the end of a randomly assigned branch. In darkness (0.0007 lux), foraging ants were allowed to locate the food source and were subsequently marked with grey acrylic paint while feeding. Unmarked ants were removed from the maze and marked ants allowed to return to the nest. Fresh paper was placed on the T maze to remove any pheromone present and the binary choices made by returning marked ants at the T junction were recorded. In addition, unmarked naïve ants were also allowed onto the maze, and their choices recorded. Ten colonies were used in this experiment, six of which were also used in experiment 2.

Statistical Analysis

Data for the pheromone deposition frequency were found to be zero inflated so we consequently chose to use the MCMCglmm package (Hadfield, 2010) implemented in R v. 2.14.2 (R Core Team, 2012) using the zipoisson family function. Uninformative prior distributions were used for fixed effect parameters with a mean of 0 and a large variance of 10^8. Priors for the variance components were inverse-Wishart distributed with the degree of belief parameter (n) set at ¼ 0.01 and variance (V) limited to 1. Each model was run for 120,000 Markov chain Monte Carlo (MCMC) simulation iterations with a burn-in of 40,000 iterations and a thinning interval of 10 iterations. Autocorrelation between successive iterations was low (<0.05). Maximal models were created and non-significant fixed effects were sequentially removed from the model. Models were compared using the deviance information criterion (DIC). The fixed effects included light treatment (levels of bright, moderate and darkness) and journey (levels of towards nest (1 & 2) & towards food source) while colony and date were used

as independent random effects. Mean parameter estimates and 95% credible intervals were constructed and are reported in the results; where estimates do not range over zero, the parameter is deemed to be significant.

Data from experiments 2 and 3 were analysed using generalised linear mixed-effect models (GLMM) with binomial errors in R v.3.4.1 (R Core Team, 2012). Models were fitted using the lmer function (Bates, Mächler, Bolker, & Walker, 2015). Following Forstmeier & Schielzeth (2011), models were constructed based on *a priori* expectations. Differences in choice behaviour for the null hypothesis were calculated using binomial tests. In experiment 3 the null hypothesis is 0.5 (random choice). In experiment 2, we compared the choice of the ants in the presence of pheromones (trained ants - information conflict) to the choice they would have made in the absence of pheromones (naïve ants - no information conflict). Data from experiment 3 provide a null hypothesis baseline for the behaviour of trained ants in darkness (0.46 do not follow their memory). The null hypothesis for bright light can be taken from Grüter et al. (2011) as 0.25. As no empirical data are available for providing a null hypothesis level for moderate light, we took an intermediate value between 0.46 and 0.25 as the null hypothesis: 0.36.

We found that naïve ants followed pheromone trails while trained ants in the dark did not, although the pairwise comparison between these groups was non-significant (see results). However, the sample size for trained ants in the dark was much lower than for naïve ants (59 vs 147 ants). Thus, to test whether the difference in pheromone following found in these two groups was due to lower power in the dark-trained ant group, we ran a power analysis. To do this, we drew 1000 random subsamples of 59 individuals (the sample size of the dark trained group) from the naïve group data, and ran an identical binomial test on each subsample to that carried out on the dark trained ants. We could then calculate the average number of times these smaller subsamples were significantly different from chance. This result was then corrected for multiple testing by subtracting the expected number of false-positives.

Ethical note

Lasius niger is not a threatened species and no license is required for work with them. Only workers from mature colonies were collected, without destroying the mother colony. *Lasius niger* colonies easily withstand food deprivation of up to a week with no ill effects. Tested workers which could not be returned to the colony were rapidly killed by freezing to minimise distress.

Results

Experiment 1: Does the frequency of pheromone deposition change with luminance?

Overall, as light level drops from bright to moderate, pheromone deposition significantly increased from 0.45 depositions per passage to 0.76 [parameter estimate = 1.477, 95% CI = (2.77,0.122); near darkness vs bright; parameter estimate = 1.206, 95% CI = (2.27,0.167), Fig. 2]. However, pheromone deposition did not continue to increase when light levels were further reduced from 10 lux to 0.0007 lux [mean 0.73 depositions per passage, parameter estimate = -0.488, 95% CI = (-1.78, 0.884)]. Note that due to the lower video quality in this setting, it is possible that pheromone deposition estimates in the moderate and dark treatment are underestimates. The fact that pheromone depositions are nonetheless seen to rise as light levels fall reinforces our results, indicating that the true pattern is even stronger than the one we report.

While the pattern of increasing pheromone deposition with decreasing light intensity holds over all three visits, the specifics differ. Significantly more deposits were made on the first journey back to the nest under the moderate light level compared to the other two light treatments [Moderate vs Bright; 0.99 vs 0.39, parameter estimate =2.18, 95% CI = (0.605, 3.71), Moderate vs Darkness; 0.99 vs 0.39, parameter estimate = 2.31, 95% CI = (1.05, 3.6), Fig. 3a]. Of particular note is the significantly greater number of depositions on the return journey from the nest to the food source in the dark, when compared to either moderate or bright-light levels [Dark vs Moderate; 0.81 vs 0.41, parameter

estimate = 2.25, 95% CI = (3.51, 0.88), Dark vs Bright; 0.81 vs 0.18, parameter estimate = 2.18, 95% CI = (3.97, 0.6) Fig. 3b]. On the second return journey to the nest, pheromone deposition was almost one deposition per passage in both darkness and moderate light, but in each case was found not to differ significantly from 0.77 deposits per passage found in bright light conditions [Dark vs Bright; 0.96 vs 0.77, parameter estimate = -0.462, 95% CI = (-2.21, 1.16), Moderate vs Bright; 0.96 vs 0.77, parameter estimate = -0.834, 95% CI = (-2.37, 0.884) Fig. 3c]. There was no significant difference in the rate of pheromone deposition between the two return journeys to the nest in either the bright or moderate light conditions [Bright; 0.39 vs 0.77; parameter estimate = 0.565, 95% CI = (-0.842, 1.73), moderate; 0.99 vs 0.96, parameter estimate = -0.174, 95% CI = (-1.35, 1.02)], but in the dark pheromone deposition significantly increased on the second return journey [darkness; 0.39 vs 0.96, parameter estimate = 1.07, 95% CI = (0.085, 1.93)].

Experiment 2: Effect of illumination on information use

The proportion of ant foragers following the pheromone trail rather than their route memories increases with decreasing light intensity (Fig. 4a). Under bright light only 28% of ants chose the pheromone treated branch, significantly less than the 61% seen in darkness (Z = -3.56, P = <0.001). More ants followed the pheromone trail in darkness than the 44% in moderate light (Z = 1.9, P = 0.059), and in moderate light vs bright light levels (Z = 1.78, P = 0.076), but these trends were not significant. Naïve ants followed the pheromone trail at the highest rate, which was significantly more than ants in bright and moderate light levels (vs bright, Z = 5.51, P < 0.001, vs moderate, Z = 3.70, P < 0.001), but not different to ants in darkness (Z = 1.45, P = 0.15). The random effect of colony contributes very little to the overall variance (<0.1%). Ants in the bright light treatment significantly preferred to follow their memories (exact binomial test with null hypothesis 0.5, 16/59, P < 0.001), and their behaviour does not differ from ants in a non-conflict situation (binomial test, null hypothesis 0.25, P = 0.76). The decisions of ants under moderate light and darkness did not significantly differ from random (moderate light: 25/58, P = 0.36, near darkness: 36/60, P = 0.16). However, for ants in darkness this represents an increased reliance on trail pheromones, as ants follow their memory significantly less

often than ants in darkness in a non-conflict situation (null hypothesis 0.46, P = 0.037). Ants at moderate light levels behaved as we would predict in a non-conflict situation (null hypothesis 0.35, P = 0.22). Naïve ants significantly preferred to follow the pheromone-marked path (106/148, $P < 0.001$).

We interpret these results as ants following a 'copy when uncertain' rule (see discussion). These results rule out an otherwise plausible alternative explanation, which is that as light levels decrease ants make more errors. This is ruled out because the excess proportion of ants not following their memory is significantly above the null hypothesis rate in the darkness treatment (0.61-0.46 = 0.15) but not in the moderate light (0.44-0.35 = 0.09) and bright light (0.28-0.25 = 0.03) treatments. If error rates were driving the low memory following in the darkness treatment, we would expect the behaviour of ants to match the null hypothesis rate.

The above pairwise comparison between naïve ants and darkness-trained ants indicated that pheromone following is not significantly different between the two groups. However, our subsequent power analysis found that, when the sample size of the naïve group was made identical to that of the darkness-trained group (59), the naïve ants' decisions were still significantly different from random 92.1% of the time. This implies that the difference between the naïve group and the dark-trained group, although not found to be significant in the main model, is in fact real, with only a c. 8% chance that it is not.

Experiment 3: Is memory based solely upon visual cues?

Ants which had made one visit to a food source at the end of a T-maze under 0.0007 lux did not perform better than naïve ants when choosing a branch at the bifurcation (Fig. 4b). While 48% of naïve ants chose the branch to the food, only 54 % of ants with a memory made the correct decision (Z= 0.99, P = 0.31). The random effect of colony and date contributed very little to the overall variance (<0.1%).

Discussion

Light levels have a large effect on the way in which *Lasius niger* foragers make use of, and deposit, pheromone trails. As previously reported (Aron et al., 1993; Czaczkes et al., 2018; Grüter et al., 2011), when route memories (private information) and pheromone trails (social information) conflict in bright light (3200 lux), *L. niger* foragers mostly follow their route memories. However, we found that as light levels decreased to dusk-like levels (10 lux) and on to darkness (0.0007 lux), the rate at which ants relied on private information decreased. When in darkness c. 60% of ants followed the pheromone trail, showing a significantly greater tendency to choose the non-memory path (Figure 4A) compared to ants not under information conflict (figure 4B). A shift in cue reliance due to light levels has been previously reported in field observations on two Formica species, *F. polyctena* (Beugnon & Fourcassie, 1988) and *F. nigricans* (Rosengren, 1977), but our study is the first to demonstrate this under stringently controlled laboratory conditions. This behaviour is consistent with ants following a 'copy when uncertain' strategy, in which reliance on social information increases as the quality of private information decreases. 'Copy when uncertain' is an adaptive information use strategy in many situations, and is employed by vertebrates in a variety of situations (Galef Jr., Dudley, & Whiskin, 2008; Jones, Ryan, Flores, & Page, 2013; Laland, 2004; van Bergen, Coolen, & Laland, 2004). Recently, behaviour consistent with 'copy when uncertain' has been described in the behaviour of *Temnothorax* rock ants during house-hunting, where informed ants rely more on social information about nest quality when their private information is uncertain (Stroeymeyt et al., 2017). Bumblebees in a foraging context have also been reported to 'copy when uncertain', being more likely to land next to bee models in uncertain environments (Smolla et al., 2016). Ants have also been reported to shift their reliance from visual to non-social odour cues in response to changes in light levels; when trained to locate food using both non-social scent cues (e.g. onion) and visual cues, various *Myrmica* species preferentially follow visual cues when olfactory and visual cues conflict. However, at lower light levels their preference shifts towards a reliance on

389 olfactory cues, in some cases even when light levels are at a moderate level of 110 lux (Cammaerts &
390 Rachidi, 2009; Cammaerts, Rachidi, Beke, & Essaadi, 2012; Cammaerts, 2012).

391 The rate of pheromone following in naïve ants agrees well with previous data (Czaczkes,
392 Castorena, Schürch, & Heinze, 2017; Grüter et al., 2011), but the pheromone following rate of ants
393 trained in darkness is lower, and is not different from chance. However, we were able to rule out the
394 possibility that our results are driven by decreasing light levels increase error rates, rather than
395 increasing pheromone following. We are thus confident that our results reflect a real shift in
396 information use. It is nonetheless important to note that in this study ants were both trained and
397 tested at the same light levels. This means that we cannot know the source of the reduction in private
398 information quality. Ants may acquire less directional information in the dark, or they may be less able
399 to use it. It is also possible that the ants can acquire and utilise directional information in the dark, but
400 choose not to use it. We consider this unlikely, however.

401 Our power analysis indicated that the difference in pheromone following behaviour of naïve
402 ants and darkness trained ants is real. The lower pheromone following rates of pheromone following
403 in darkness trained ants is likely to be driven by remaining conflict from memories. An alternative
404 explanation is that the ants were in a wrong-task state (Czaczkes et al., 2017; Roces, 1993); naïve ants
405 may be actively scouting (exploring or otherwise ready to make use of social information), while
406 experienced ants may be in a foraging state, attempting to exploit private information even if it is not
407 there, and disregarding social information. However, Czaczkes et al. (2017) showed that task state
408 does not in fact influence pheromone following. As both naïve ants and ants in a different task states
409 but with no directional information do follow pheromone, we can be confident that the trained ants
410 in darkness had access to the trail-pheromone information.

411 Ants seem to learn very poorly in darkness, or not at all (54% correct choices), while they are
412 very capable of doing so on almost identical mazes in lit conditions (c. 75% correct choices, (Grüter et
413 al., 2011; Oberhauser et al., 2018)). It is possible that, given more visits, ants would learn to navigate

414 the maze reliably using idiothetic (internal body movement) cues, as has been shown in other ant
415 species (Macquart, Latil, & Beugnon, 2008). It is worth noting that visual cues are in principle not
416 required for navigation by path integration (Collett & Collett, 2000), where an odometer linked to any
417 directional cue can be used to estimate displacement from a starting location. Magnetic cues have
418 been shown to be used for navigation by several animals, including ants, especially when other cues
419 are unavailable (Banks & Srygley, 2003; Lohmann, Lohmann, & Putman, 2007). Path integration is
420 usually used as the initial navigation mechanism by desert ants, before route-based navigation
421 memories are formed (Collett et al., 2013). If the same is true for *L. niger*, this would imply that *L. niger*
422 require visual directional cues to perform path integration effectively.

423 Pheromone deposition in *L. niger* is very variable between workers, with most ants depositing
424 nothing, and some depositing many dots per passage. However, as pheromone depositions are
425 cumulative, it is the mean pheromone deposition rates that are relevant to the colony. Inspection of
426 these showed that rates of pheromone deposition varied strongly with light levels. Broadly, over all
427 visits, ants deposited about 40% less pheromone at the bright light than in the moderate light level or
428 darkness (Fig. 2). Along with the poor navigational performance of ants in darkness, this supports the
429 assertion that foragers are less confident of their location in moderate light levels and darkness.
430 Previous studies have demonstrated that pheromone deposition rates correlated with navigational
431 confidence. For example, Heinze & Czaczkes (2015) trained ants to make return visits to food source
432 at the end of a T-maze, as in the current experiment. They found that pheromone deposition rates of
433 outgoing ants which would go on to make a navigational error were lower than those of ants which
434 would make a correct decision. This implies that the ants had some measure of their own uncertainty,
435 or are vacillating between an informed and a naïve state. Once such 'mistaken' ants finally find the
436 food source, they increase their pheromone deposition rates on their return journey to the nest
437 (Czaczkes et al., 2013; Czaczkes, Weichselgartner, Bernadou, & Heinze, 2016; Czaczkes & Heinze,
438 2015), presumably in an effort to provide more information on difficult-to-navigate routes. We
439 suggest that the similar increase in pheromone deposition in dim light and darkness we report here is

440 also best understood as an effort to increase information availability under challenging navigation
441 conditions. It is important to note, however, that in our experiment all ants were tested during their
442 circadian day. It is possible that pheromone deposition in the dark when ants 'expect' it to be dark
443 may differ from what is reported here.

444 A more complex picture emerges when we examine each journey of the ants separately. On
445 the first return to the nest, ants in moderate light deposit about 45% more pheromone than ants in
446 darkness, and also 20% more than ants in bright light (Fig. 3). We interpret this again in terms of
447 certainty and information provision (Czaczkes et al., 2013; Czaczkes & Heinze, 2015). Thus, ants in
448 moderate light can be interpreted to sense that more information is needed compared to ants in
449 bright light, and therefore provide this information. Ants in darkness deposit very little pheromone,
450 but this is not surprising – ants which are lost or unexpectedly leave a pheromone trail deposit little
451 or no pheromone (Czaczkes, Grüter, Jones, & Ratnieks, 2011), TJC pers. obs). Surprisingly, on their
452 return to the food source, outgoing ants in darkness deposit on average almost 78% more pheromone
453 than ants returning in bright light. This was unexpected, given that they apparently cannot know
454 exactly where they are going (Fig. 4B). However, as some pheromone and home-range markings had
455 already been deposited, this may act as a reassurance that ants are on the right path (Czaczkes et al.,
456 2011; Devigne, Renon, & Detrain, 2004; Wüst & Menzel, 2017). Given that they are on the right path,
457 reinforcing the pheromone signal provides more information in darkness, where visual information is
458 lacking. Finally, on the second return to the nest, ants consistently deposit a high amount of
459 pheromone at all light levels. We interpret this as all ants, having found food twice in quick succession,
460 being confident enough of their location to recruit strongly to the food source.

461 How animals strategically use and deploy information has been the subject of intense research
462 (Dall et al., 2005; Grüter & Leadbeater, 2014; Laland, 2004). The information richness of an
463 information source is predicted to be a strong driver of information source use (von Thienen et al.,
464 2016). Studying visual information use under varying light levels provides a powerful and ecologically

relevant means of manipulating information richness. By taking advantage of this, we show that information use during *Lasius niger* foraging is consistent with a 'copy when uncertain' strategy. We also demonstrate that ants vary their provision of an alternative information source as their primary information source becomes less informative.

Author contributions

SJ and JB conceived of the study. SJ planned the study. SJ and TJC performed the statistical analysis. FBO performed the power analysis. SJ, AG, and EG collected the data. TJC, SJ and JB wrote the manuscript. All authors gave final approval for this work.

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682

683 # Figure captions
684

685 **Fig 1. (a)** Experimental design used to measure trail pheromone deposition frequency by foraging ants
686 under three different lighting regimes (darkness, moderate and bright). For each treatment frequency
687 of deposition was recorded for the observed section (lined area) for three journeys: the first return
688 journey to the nest, the first outward to the food source and the second return to the nest. Each
689 experiment involved 8-12 ants that were marked with paint while feeding on the sugar solution for the
690 first time. **(b)** Experimental design used to acquire trail pheromone for subsequent conflict situations. A

691 section of paper (X) was partially covered by a segment (Y) and ants were allowed to forage on a 1M
692 sugar solution. Pheromone deposition on the uncovered part of section X was monitored until 35-40
693 deposits were reached, after which foraging was stopped and section X, was transferred to a second T
694 maze for experiment 2**.** Segment Y was discarded, resulting in a pheromone-free part of section X. **(c)**
695 Experimental design used for experiment 2. Ants were allowed to locate a 1M sugar solution on a
696 randomly chosen branch. While feeding, ants were marked with a paint dot and allowed to return to the
697 nest. Section X was then transferred to the bifurcation so that marked returning ants were faced with a
698 conflict between their route memory and trail pheromone. Decisions were recorded once an ant passed
699 either of the two decision lines. Naïve ants with no memory were also tested to determine their response
700 to pheromone alone, and as a control for any side bias.
701
702 **Fig 2**. Violin plots show the number of pheromone deposits per passage for the 3 different light levels
703 (Dark, Moderate and Bright) for the three journeys combined. Circles are individual data points, red
704 diamonds denote means. Different letters (a, b) signify significant ($P < 0.05$) differences between groups.
705 The figure is cropped at 8 depositions for clarity, omitting one data point in the 'Dark' treatment. A slight
706 jitter was added to the individual data points to allow individual points to be distinguished.
707
708 **Fig 3**. Violin plots show pheromone depositions per passage for each of the three light levels for the
709 three separate journeys: **(a)** first return to nest **(b)** outgoing to food and **(c)** second return to nest. Circles
710 are individual data points, red diamonds denote means. Different letters (A,B,C,D) signify significant ($P < 0.05$) differences between groups. A slight jitter was added to the individual data points to allow
712 individual points to be distinguished. Not that one datapoint in (c) dark treatment was omitted for clarity,
713 as in figure 2.
714
715 **Fig.4. (a)** Histogram showing the proportion of ants choosing the branch of a T maze treated with trail
716 pheromone when presented with a conflict between their own route memory (acquired from one visit)
717 and trail pheromone, under three different lighting regimes (Darkness, Moderate light, and Bright Light).
718 A comparison is also made to naïve ants with no route memory. **(b)** Histogram showing the proportion
719 of ants choosing the branch of a T maze that leads to a food source in darkness. Different letters (A,B,C,
720 given above each bar) denote significant differences, derived from post-hoc analysis of GLMMs. *** and

* denote group different from the null hypothesis as $P < 0.001$ and $P < 0.05$ respectively and n.s. denotes not significantly different from the null hypothesis, derived from binomial tests. Null hypothesis (dashed) lines represent the expected behaviour of the ants given no conflict. This is 0.5 for naïve ants and trained ants in B, 0.46 for ants in darkness in A (the reciprocal of trained ants in B), 0.25 for ants in bright light (based on Grüter et al. 2011), and 0.35 for ants in moderate light (an intermediate value between 0.46 and 0.25). Error bars represent 95% confidence intervals. Percentages in bars give the proportion of ants following the pheromone trail (A) or choosing the arm where food was encountered (B), and numbers denote group sample size.